

Amendments to the Specification:

Please replace the paragraph beginning at page 4, line 20, with the following rewritten paragraph:

31 -- Figures 1A-1C illustrate the full length wild-type MerR protein, as the ~~monomeric metal binding domain~~, monomer, the dimeric coiled-coil metal binding domain and the mercury-bound dimeric metal binding domain, respectively. In each, the tube represents the coiled-coil region, the small open spheres represent cysteine residues, the hatched curve is the short loop regions, the large cross-hatched circles represent all other regions of the protein, and the small black sphere represents bound mercuric ion. Dimer in solution binds one Hg ion because of the conformational change. ~~Figure 1B illustrates~~ Figures 1D-1E illustrate the chelon protein with its tandem metal binding domains. The shapes are as in Figure 1A except that the hatched curves represent the nonwild-type linker residues which allow association of the coiled-coils and stabilize the protein.--

[Replace the paragraph beginning at page 8, line 20, with the following rewritten paragraph:]

32 -- The present work provides the first demonstration that an independent sub-domain of a naturally occurring protein can be incorporated in a heavy metal sequestration protein which binds ~~cognate~~ thiophilic metal ions with ~~high specificity and~~ high affinity. Previously described metal binding proteins are those which bind divalent "beneficial" metal ions, e.g., nickel, copper and zinc. Generally for those proteins, the affinities and specificities for heavy metals are lower, although some forms of metallothionein bind divalent cadmium ions. The previously known metal binding proteins are subject to having the metal ligand competed off by naturally occurring thiols such as glutathione and cysteine. The relatively low affinities reflect the physiological roles of these proteins in serving as chaperones for the metal ions en route to the active sites of enzymes

32 out or structural sites of DNA binding proteins. The intact MerR protein and its metal binding domain have much higher affinity (about 9 orders of magnitude) than natural thiols such as glutathione or cysteine and nonphysiological thiols such as 2-mercaptoethanol [Zeng et al. (1998) supra]. The wild-type MerR protein binds mercuric ion with an affinity of 10^{-9} M even in the presence of millimolar quantities of thiols, and it binds divalent cadmium and divalent zinc ions a hundred-fold and thousand-fold less well.--

[Replace the paragraph beginning at page 12, line 14, with the following rewritten paragraph:]

33 -- MerR or chelon proteins can be readily engineered to facilitate purification and/or immobilization to a solid support of choice. For example, a stretch of 6-8 histidines can be engineered through mutagenic polymerase chain reaction or other recombinant DNA technology to allow purification of expressed recombinant protein over an nitrilotriacetic acid (NTA) column using commercially available materials. Other oligopeptide "tags" which can be fused to a protein of interest by such techniques include, without limitation, strep-tag (Sigma-Genosys, The Woodlands, TX) which directs binding to streptavidin or its derivative streptactin (Sigma-Genosys); a glutathione-S-transferase gene fusion system which directs binding to glutathione coupled to a solid support (Amersham Pharmacia Biotech, Uppsala, Sweden); a calmodulin-binding peptide fusion system which allows purification using a calmodulin resin (Stratagene, La Jolla, CA); a maltose binding protein fusion system allowing binding to an amylose resin (New England Biolabs, Beverly, MA); and an oligo-histidine fusion peptide system which allows purification using a Ni^{2+} -NTA column (Qiagen, Valencia, CA).--